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THE ORGANIZATION OF THE SUPRACHIASMATIC CIRCADIAN PACEMAKER OF THE RAT AND ITS REGULATION BY NEUROTRANSMITTERS AND MODULATORS (UNC)

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This research addresses the cellular organization and regulation of a biological clock that controls daily (circadian) rhythms of behavior (e.g., performance), physiology and metabolism in mammals. This clock, located in the brain's suprachiasmatic nucleus (SCN), can be removed in a slice of hypothalamus, maintained in a life support system for up to 3 days and studied directly. Using this approach, progress in year 1 of this award has been made in 1) localizing time-keeping properties within the SCN of rat, 2) establishing the regulatory role of serotonin, a neuromodulatory input from the brain's arousal center in the raphe nucleus, and 3) examining the release of excitatory amino acids from the optic tract in the region of the SCN. This project involves both individual and interactive research projects at the University of Illinois and the USAF SChool of Aerospace Medicine.

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THE ORGANIZATION OF THE SUPRACHIASMATIC CIRCADIAN PACEMAKER OF THE RAT AND ITS REGULATION BY NEUROTRANSMITTERS AND MODULATORS
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RESEARCH OBJECTIVES

The suprachiasmatic nucleus (SCN) of the hypothalamus is a circadian pacemaker that serves a well-defined, critical role in the generation and entrainment of daily rhythms of physiological, metabolic and behavioral functions in mammals. The ensemble of SCN neurons generates near 24-hr rhythms of electrical activity and vasopressin secretion that time the oscillations in mammalian circadian rhythms. Timing of SCN rhythms is reset by changes in environmental lighting, which can affect the SCN through inputs from the retina, intergeniculate leaflet or the raphe. However, little is known about the way in which the neuronal components of the SCN are organized to carry out time-keeping or to analyze phase-resetting information. This study seeks to determine the functional organization of the SCN by electrophysiological analysis of regional distribution of pacemaking properties and responses to extrinsic and intrinsic neurotransmitters and modulators.

We are using the rat hypothalamic brain slice to study the functional organization of the SCN directly. Our work has established that circadian pacemaking and resetting properties are endogenous to the SCN and can be studied in vitro. In the studies undertaken in year 1 of this award, the circadian rhythm of SCN electrical activity was recorded extracellularly in intact and microdissected slices of rat hypothalamus. Persistence of a rhythm in microdissected subregions was determined. The neuromodulator serotonin or its agonists were applied focally with micropipette. The phase of the ensemble electrical activity rhythm was assessed for 24-48 hr after treatment. Additionally, Dr. Rea's lab at the USAF-SAM has begun to investigate release of excitatory amino acids in the SCN region upon electrical stimulation of the optic nerve.

The main hypotheses tested in this study include: 1) pacemaking properties are distributed throughout the SCN; 2) neuromodulators from an identified input (serotonin from the raphe) are effective phase-shifting agents during the circadian day; and 3) light information carried by the retinohypothalamic tract affects the SCN via excitatory amino acids.

The long-term goal of these studies is to understand how neurons of the SCN are organized to generate a 24 hr biological clock and what role specific neurotransmitters and modulators play in the pacemaking and resetting process. Because the SCN integrate most circadian behaviors and metabolic fluxes, this study has basic relevance to understanding circadian dysfunction induced by transmeridian travel and sustained, irregular work schedules, with possible application to improving human performance under conditions that induce circadian desynchronization.

PROGRESS TOWARD SPECIFIC AIMS:

The following specific aims, formulated in terms of hypotheses to be tested, from the original proposal have been addressed in the first year of the award and substantial progress has been made toward each. A summary of the rationale of the experiments, the methodological approach, the results and the interpretation of each follows.

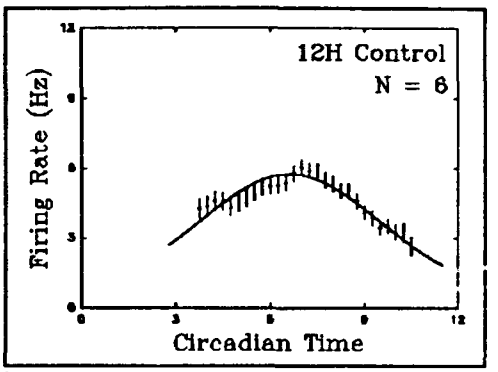


Figure 1a. 12 hour control SCN. Sliding window averages and fitted curve show a mid-day peak in firing rate.

1) Pacemaking properties are distributed throughout the SCN. This hypothesis is being tested by microdissecting the SCN into the well described dorsomedial (DM, source of efferents) and ventrolateral (VL, region that receives afferents) subregions and measuring the ability of each part to generate a circadian rhythm of neuronal activity. Activity is compared with that in the same subregions of intact SCN.

We have found that both the VM and DL regions oscillate in the intact SCN, as well as in single SCN whose connections to the other member of the bilateral pair in the brain slice have been severed by cutting ventral to the third ventricle which bisects the slice. Subsequent experiments have assessed the firing pattern after bisection of the slice and then hemisection of the SCN into DM and VL regions. Measurements were made on these regions over 12 and 24 hr periods. Results were analyzed both empirically and by statistical curve fitting. These experiments were carried out by Thomas Tcheng, a Neuroscience program graduate student in my laboratory.

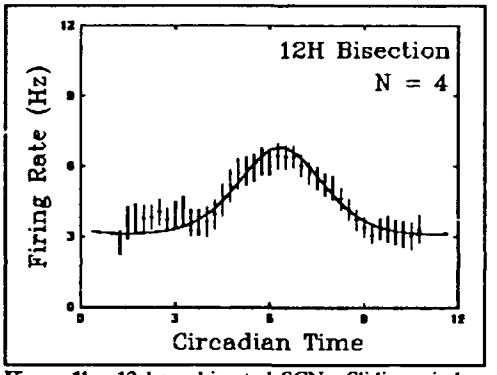


Figure 1b. 12 hour bisected SCN. Sliding window averages and fitted curve show a mid-day peak in firing rate similar to control.

METHODS

Hypothalamic brain slices containing 500 μm coronal sections of the paired SCN were prepared and left intact, bisected, or hemisected. Control slices were left intact. Slices were bisected by cutting the fibers connecting the paired SCN, isolating each nucleus from the other. Slices were hemisected by first bisecting the SCN, then cutting the SCN into dorsomedial and ventrolateral regions. Isolated regions were examined for evidence of an electrical circadian rhythm. Two groups of experiments were performed with different durations: 12 and 24 hours. Control, bisected, and hemisected SCN were included in the 12 hour group. Entire and hemisected SCN comprised the 24 hour group.

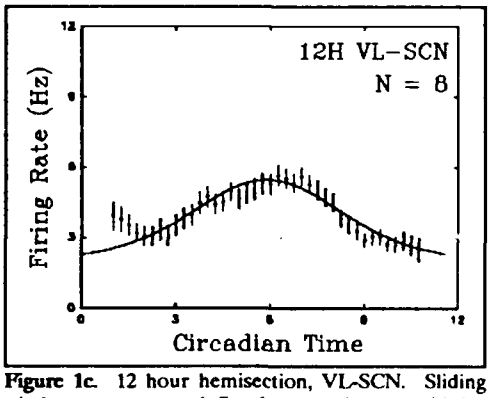


Figure 1c. 12 hour hemisection, VL-SCN. Sliding window averages and fitted curve show a mid-day peak similar to control and bisection.

BRAIN SLICE. Coronal brain slices containing a 500 μm section of the SCN are prepared during the day from 2-3 month old Long-Evans rats housed on a 12:12 LD (light/dark) cycle. Slices are perfused in a brain slice chamber and situated at the liquid-gas interface. The slices are perfused with a minimal medium containing Earle's Balanced Salt Solution, supplemented with 24.6 mM glucose and 26.2 mM $NaHCO_3$, saturated with $95\%O_2/5\%CO_2$, and maintained at 37°C and a pH of 7.40. The slices remain illuminated throughout an experiment.

ELECTRICAL RECORDING. Average firing frequencies for individual neurons are used to gain evidence for a circadian rhythm of electrical activity. Spontaneous neuronal firing of single neurons is recorded extracellularly. Individual neurons are identified by their firing pattern and action potential waveform and an average firing rate is calculated over a minimum of two two-minute periods. This procedure is repeated for as many cells as possible for the duration of a recording session, usually 12 or 24 hours. Recording sites are arbitrarily chosen to reflect a random sample of neurons within the isolated region being studied.

DATA ANALYSIS. Circadian phase of the SCN can be determined by empirical analysis of sliding window averages. Raw data from individual cells are grouped into two hour bins, incremented in 15 minute steps, from which average firing rates and standard errors are calculated. This treatment acts as a low-pass filter, smoothing out high-frequency variability in the raw data and preserving the low-frequency oscillation. The phase of the electrical rhythm is determined by visually estimating the time-of-peak from the sliding window averages plotted against circadian time. The normal time-of-peak is CT 6.9, or 6.9 hours after the lights are turned on in the colony.

For statistical analysis, a parametric curve is fitted to raw data and several descriptive statistics are extracted from the equation after curve-fitting. The presence of significant differences between experimental groups is determined using one-way ANOVAs. These differences are then quantified using one- or two-tailed t-tests.

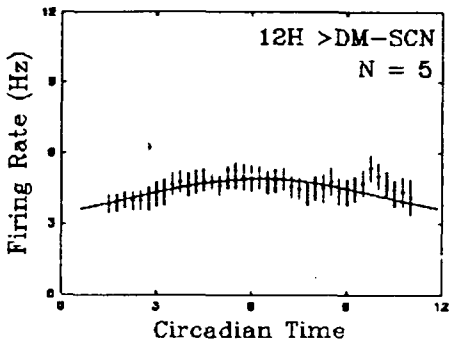


Figure 2a. *12 hour ventrolaterally biased* hemisection, DM-SCN. The amount of SCN in the DM-SCN is largest. Sliding averages and the fitted curve show evidence of a dampened mid-day peak.

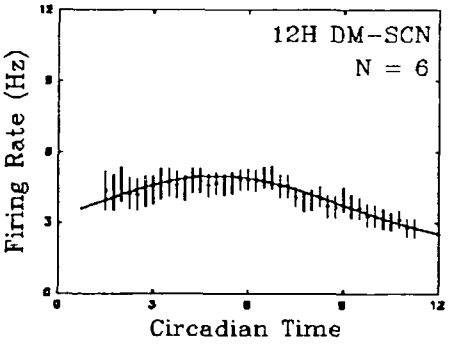


Figure 2b. 12 hour hemisected SCN, DM-SCN. Sliding window averages and the fitted curve show some evidence of a mid-day peak.

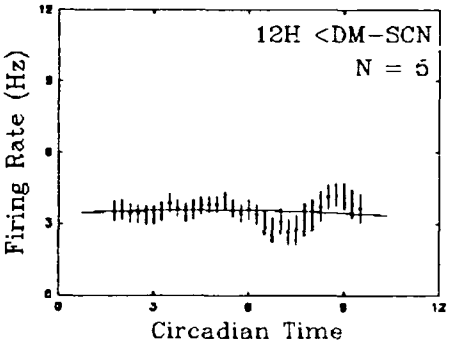


Figure 2c. 12 hour dorsomedially biased hemisection, DM-SCN. There is the least SCN in the DM-SCN after this treatment. Sliding window averages and the fitted curve show very little evidence of an oscillation.

RESULTS

Empirical analysis suggests degradation of the electrical rhythm mainly in the DM-SCN after hemisection. Figures 1a, 1b, and 1c show mid-day peaks in electrical activity from 12 hour control, bisection, and VL-SCN hemisection experiments. Progressive reduction of the DM-SCN as shown in Figures 2a, 2b, and 2c degrades the mid-day peak.

Figures 3a, 3b, and 3c show 24 hour control, hemisected VL-SCN, and hemisected DM-SCN experiments. The control group shows a broad nighttime trough and a sharp daytime peak. The hemisected VL-SCN shows a similar, though somewhat disrupted, pattern with a shorter nighttime trough and a broader daytime peak with multiple inflections. The hemisected DM-SCN shows a less coherent pattern. The sliding window averages suggest short-period oscillation while the fitted curve suggests an extremely dampened oscillation.

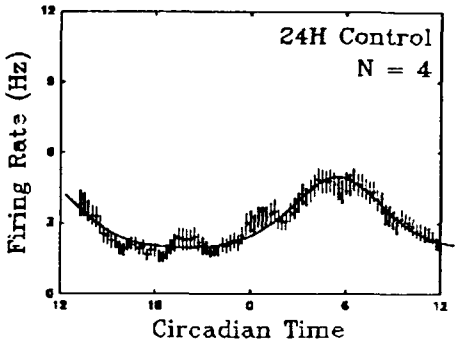


Figure 3a. 24 hour control. Both the sliding window averages and the fitted curve show a nighttime trough and a mid-day peak.

DISCUSSION

These findings indicate that isolated regions of the SCN are capable of retaining some residual pacemaker activity. Coronal sections of the SCN contain a complete functional pacemaker. The isolated VL-SCN also appears to contain a pacemaker, although it may be impaired by surgical isolation. The presence of a functional pacemaker in the isolated DM-SCN is questionable. It's electrical activity is very different from a normal circadian rhythm, but it does appear to have some pattern. One can conclude from this research that integration of various neural assemblies within the SCN are necessary for normal pacemaker function. The essential components of the pacemaker appear to be primarily localized in the VL-SCN.

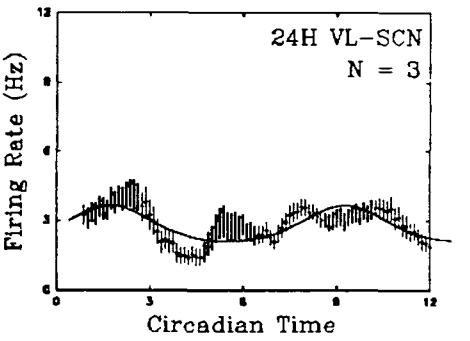


Figure 3b. 24 hour hemisected SCN, VL-SCN. Sliding window averages suggest a short trough at night and multiple peaks during the day. The fitted curve suggests a shortened period.

FUTURE RESEARCH

Two projects related to this research are in the early stages of experimentation. The first is to construct a descriptive and predictive model of the circadian pacemaker, incorporating the findings of our lab and others in the field. STELLA, a computer modelling program will be used to model the biochemical pathways underlying the circadian pacemaker. The second project will employ optical recording techniques to study dynamic relationships within the SCN. This project will attempt to relate multiple cell activity to the neuroanatomical and immunohistochemical organization of the SCN. This integrated analysis will provide further insight into pacemaker function.

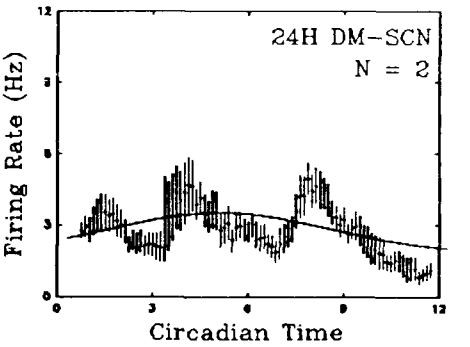


Figure 3c. 24 hour hemisected SCN, DM-SCN. The two analytical techniques do not agree well. Sliding window averages suggest short period oscillation. The fitted curve shows little evidence of an oscillation.

2) Serotonin, a neuromodulator contained in afferents from the raphe, is an effective phase-shifting agents during the circadian day. This hypothesis was tested by evaluating the effects upon the rhythms of neuronal activity of focal application of a 30 *fl* droplet of 10^{-6} M serotonin upon the SCN region receiving inputs from the raphe. We also have begun to evaluate the specificity of the serotonin effect and receptor subtype with specific 5-HT agonists. These experiments are begin conducted by Marija Medanic, a Physiology & Biophysics graduate student in my laboratory.

METHODS

The SCN were isolated in a 500μm hypothalamic brain slice and maintained in a Hatton-style (Hatton, 1980) brain slice chamber for up to three days. The slices were continuously supplied with 95% O_2, 5% CO_2 and perfused with glucose and bicarbonate supplemented Earle's Balanced Salt Solution (GIBCO). The slices were treated on day 1 by localized applications of serotonin and serotonin-specific agonists (8-OH dipropylaminotetralin (8-OH DPAT) and 5-Carboxamidotryptamine (5-CT) (RBI)) (Sanders-Bush, 1988) to the ventrolateral regions of one of the SCN in the slice. A microelectrode filled with a pharmacological agent (10^{-6}M) was positioned over the desired region of the slice and a spherical drop of 10^{-11}ml was deposited on the region of the SCN that receives serotonergic inputs. Slices were treated with serotonin at 7 different time points across the circadian cycle (circadian time 0 (CT 0) being at the onset of light and continuing for 24 hours). Specific serotonin agonists were applied at CT 9, a time at which serotonin was known to affect the pacemaker, to determine the specificity and receptor subtype of the serotonin-induced phase-shifts. The effects of the treatments on the phase of the pacemaker were accessed on the second and third days *in vitro* by extracellularly recording the rhythm of neuronal activity, and comparing the time-of-peak to untreated slices. Normally, the time-of-peak between experiments is highly stable and predictable so that the peak can be used as a marker of the phase of the circadian pacemaker (Prosser & Gillette, 1989).

RESULTS

1. *Serotonin effects the SCN pacemaker directly.* Application of serotonin on day 1, at CT 7 (n=3), resulted in large phase advances (6.9 ± 0.1hr) in the time-of-peak of the rhythm of electrical activity, recorded on day 2 (Figure 4B).

2. *Serotonin permanently resets the phase of the oscillator.* Administration of serotonin at CT 7 on day 1 resulted in a 6.9hr phase-advance of the neuronal activity rhythm recorded on day 2 and day 3 (Figure 4B & C). The time-of-peak recorded on day 3 occurred at CT 0, which is nearly 24 hours after the peak on day 2, indicating that the phase change due to treatment with serotonin is a permanent one.

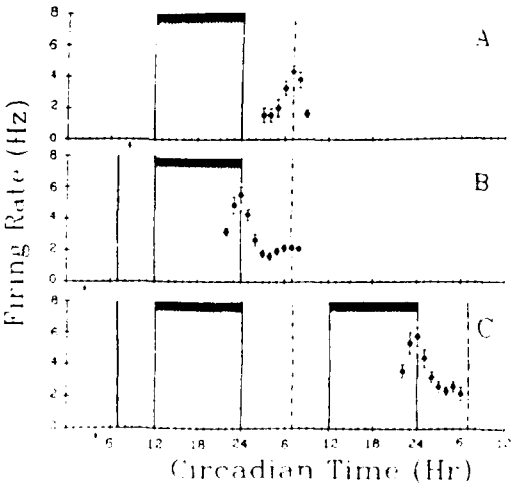


Figure 4 Serotonin induced permanent phase-shifts *in vitro*. A. Rhythm of endogenous neuronal activity recorded in untreated slices on day 2. B. Localized application of serotonin to the ventrolateral region of the SCN slice at CT 7 resulted in a 7 hour phase advance in the rhythm of electrical activity on day 2. C. Recording on day 3, in a separate experiment, following treatment with serotonin at CT 7 on day 1, indicated a 6.9 hr phase advance. This is overlapping with the mean phase advance seen on day 2. The *filled circles* represent the 2 hour means ± SEM of the recorded neuronal activity rhythm on the second and third day. The *vertical bar* indicates the time of serotonin treatment and the *dashed line* indicates the time of peak observed in untreated slices. The *stippled bar* indicates the time of the donors subjective night in the colony. *Arrows* point out the time of slice preparation.

3. *Serotonin effects the phase of the SCN in the daytime.* Treatment of SCN slices in the daytime resulted in significant advances in the phase of the rhythm of neuronal activity, with the greatest sensitivity at CT 7. When the slices were treated at time points during the night there was no significant change in the time-of peak. Figure 5 is a phase response curve that illustrates the effects of serotonin on the rhythm of electrical activity across the circadian cycle.

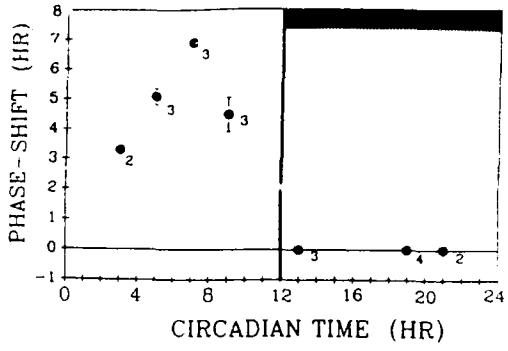


Figure 5 Phase-response curve for serotonin. The *x*-axis denotes circadian time (CT) of treatment (hr) and the *y*-axis indicates the average magnitude and direction of the treatment induced phase-shift (hr). The magnitude of the shift of the time-of-peak of the electrical activity rhythm was determined in relation to time-of-peak in untreated slices. *Filled circles* represent the mean ± SEM phase-shift. The *subscript number* is the number of experiments performed at a particular time point. The *vertical bar* denotes the time of lights-off in the colony and the *stippled horizontal bar* indicates the subjective night.

4. *The change in the phase of the pacemaker is serotonin-specific.* The specificity of the phase change induced by serotonin was accessed by treating slices with 5-CT, an agonist specific for the 5-HT_1 receptor subtype. Application of this agonist at CT 9 resulted in a 6.5±0.2hr(n=3) advance in the time-of-peak. This is a time at which serotonin induced a 4.5hr phase-advance of the rhythm of neuronal activity. Application of a microdrop of 5-CT to the SCN at CT 15 (n=2), at a point when the SCN is insensitive to serotonin, caused no effect on the time-of-peak in the next oscillation.

5. *Serotonin phase-shifts may be cAMP mediated.* To further investigate the specificity of the serotonin induced phase-shifts, slices were treated with 8-OH DPAT, another serotonergic agonist. 8-OH DPAT is specific for 5-HT_{1A} receptor subtypes, which involve a cAMP-mediated second messenger pathway. Application of 8-OH DPAT at CT 9 induced 7±0.1hr (n=3) phase advances, suggesting that serotonin induces phase-shifts through a cAMP mechanism.

FUTURE DIRECTION

Over the course of the next year we will continue the investigate the involvement of serotonin in the mammalian circadian system. The following experiments will be performed:

1. *Determination of the sensitivity of the serotonin effect.* A dose response relationship will be worked out for the point of maximal sensitivity.

2. *Examination of the signal transduction pathways underlying the serotonin-induced phase- shifts.* This involves further testing of the hypothesis that serotonin acts by a cAMP-mediated system. Specific experiments include testing the sensitivity of the serotonin stimulated phase-shifts to specific antagonists of the 5-HT_{1A} receptors, the efficacy of other receptor subtype agonists, the effect of microdrops of 5HT applied at other sites in the slice, and the effect of 5HT on cAMP levels.

3) The retinohypothalamic tract affects the SCN by excitatory amino acids (EAAs). This hypothesis was tested using the horizontal slice with optic nerves attached. First, the ability of optic nerve stimulation to induce release of excitatory amino acids from preloaded optic nerve terminals was addressed in Dr. Mike Rea's laboratory at the USAF-SAM. The results of these experiments, provided by Dr. Rea, follow.

PROGRESS

Slice Physiology

A system for the study of the neurochemistry of retinohypothalamic tract (RHT) stimulation-induced electrical activity in the SCN using the horizontal hypothalamic slice has been established and characterized. This project required the design and fabricaton of a special slice superfusion and stimulation chamber. The chamber, which is fabricated from Plexiglas, has a steady-state solution volume of 300 microliters and is jacketed to permit temperature control. Ports in the side of the chamber allow the introduction of suction electrodes at the level of the slice. The floor of the chamber is composed of Sylgard which provides a soft surface onto which the slice is secured with fine silver tacks. The chamber is mounted on the stage of a stereo-microscope and the slice can be observed during an experiment by transillumination.

The slice is totally submerged and constantly superfused with oxygenated Krebs-Ringer bicarbonate buffer. The buffer is delivered to and removed from the chamber by a multichannel peristaltic pump. The buffer enters the chamber at the level slice and is removed at a slightly higher rate from the top of the chamber. This arrangement maintains a constant rate of flow through the chamber, determined by the rate of buffer delivery (typically 0.8 ml/min), and results in a constant solution volume of 0.3 ml. An examination of the flow characteristics of the chamber using colored dyes demonstrated efficient slice superfusion and showed that the solution in the chamber is completely replaced approximately every 2 minutes. At a flow rate of 0.8 ml/min and a temperature of 37°C, the partial pressure of oxygen in the chamber is 550 mm Hg.

Electrical stimulation of one optic nerve (0.7 mA square wave pulse of 300 usec duration; 1 to 5Hz) elicits a robust field potential response (160 $\pm$ 70 uV; latency = 12 $\pm$ 1 msec) in the contralateral SCN. In the rat slice, the response is most pronounced when the recording electrode is located in the ventrolateral aspect of the SCN. The field potential is totally blocked by 1 uM TTX and requires the presence of extracellular calcium. Furthermore, the field potential is reversibly blocked by selective non-NMDA glutamate receptor antagonists such as DNQX. These results demonstrate that the slice is both structurally intact and viable, and support the theory that RHT neurotransmission is mediated by excitatory amino acids.

Stimulated Release of Excitatory Amino Acids

During all release experiments the field potential response to optic nerve stimulation was monitored continuously. Fractions were collected on ice, lyophilized, and assayed for amino acid content by reversed phase HPLC.

Initial studies of amino acid release were very encouraging. Electrical stimulation (1 Hz using a bipolar stimulating electrode) resulted in increased efflux of glutamate and aspartate from the slice. However, we were unable to consistently block the release of amino acids with TTX.

Futhermore, non-transmitter amino acids (serine and others) were released as well. These observations led us to suspect that the apparent release of amino acids might be due either to local damage of the optic nerve by the bipolar electrode or to field stimulation of the SCN. Therefore, we switched to suction electrodes, which gave better field potential responses (as high as 520 uV) and eliminated these concerns. Since switching to suction electrodes, the electrically evoked release of amino acids has been inconsistently observed. Our inability to consistently demonstrate release could be due variation in the thickness of the slice, perhaps resulting in variable reuptake efficiency. Alternatively, it is possible that the pool of releasable glutamate is too small to detect using the current methods. In an attempt to improve signal-to-noise, we are (1) preloading the terminals with [^{3}H]- glutamate, (2) attempting to block reuptake/metabolism of released glutamate by including uptake inhibitors (e.g., D-(+)-theo-3-hydroxyaspartate), and (3) experimenting with the Syrian hamster SCN which receives more robust and widespread RHT innervation. Results from these experiments are too preliminary to discuss at this time.

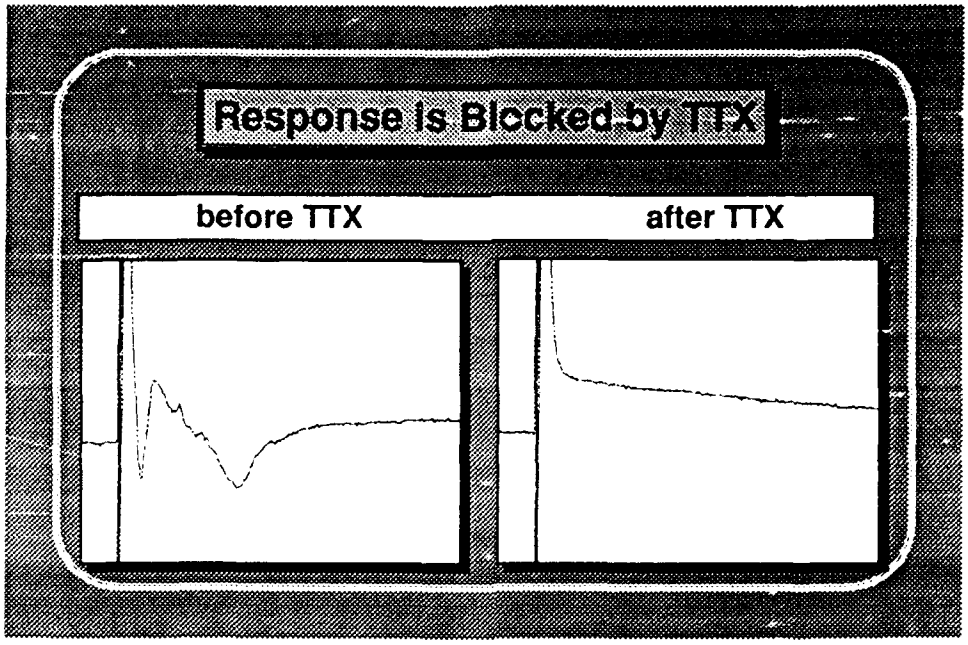
FUTURE APPROACHES

Slice Physiology

Although we have experienced some difficulty with the release protocol, slice physiology is remarkably reliable in our hands. Therefore, we will proceed with our study of the presynaptic regulation of RHT terminals, measuring the effect of $GABA_B$ and nicotinic drugs on the presynaptic membrane potential, as well as the optic nerve stimulation-induced field potential, using the sucrose-gap method described recently by King (Brain Res 527:150- 154,1990). We will study the effects of these drugs in slices prepared at six hour intervals during the circadian cycle to determine whether responses are modulated by the SCN circadian pacemaker.

Amino Acid Release

We feel that this study will ultimately provide important information concerning the regulation of RHT neurotransmission and should be pursued. Therefore, we will continue with our efforts to improve the protocol as described above. In addition, we will arrange a visit with Dr S. Y. Liou who was the primary author of the first and only published report demonstrating the release of radioactivity from hypothalamic slices preloaded with [^{3}H]-glutamate in response to optic nerve stimulation. Perhaps Dr Liou would be willing to visit our lab on his way to a future scientific meeting in the US. Finally, Dr Namboodiri at Georgetown University has agreed to assay our superfusates for N- acetylaspartylglutamate (NAAG), which has been implicated in RHT neurotransmission.


Response is Blocked by TTX
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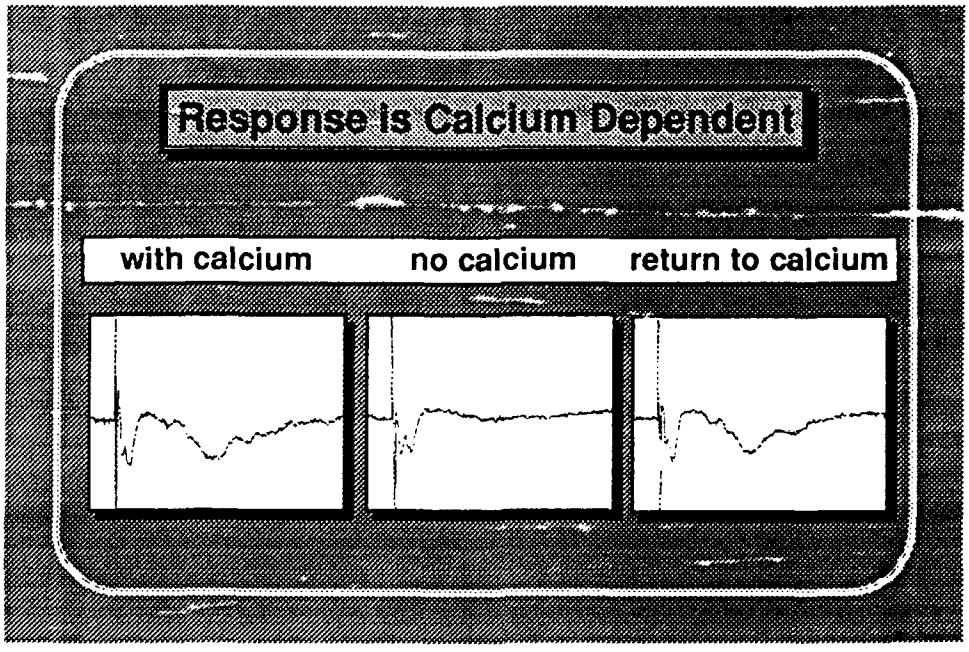
Response is Calcium Dependent
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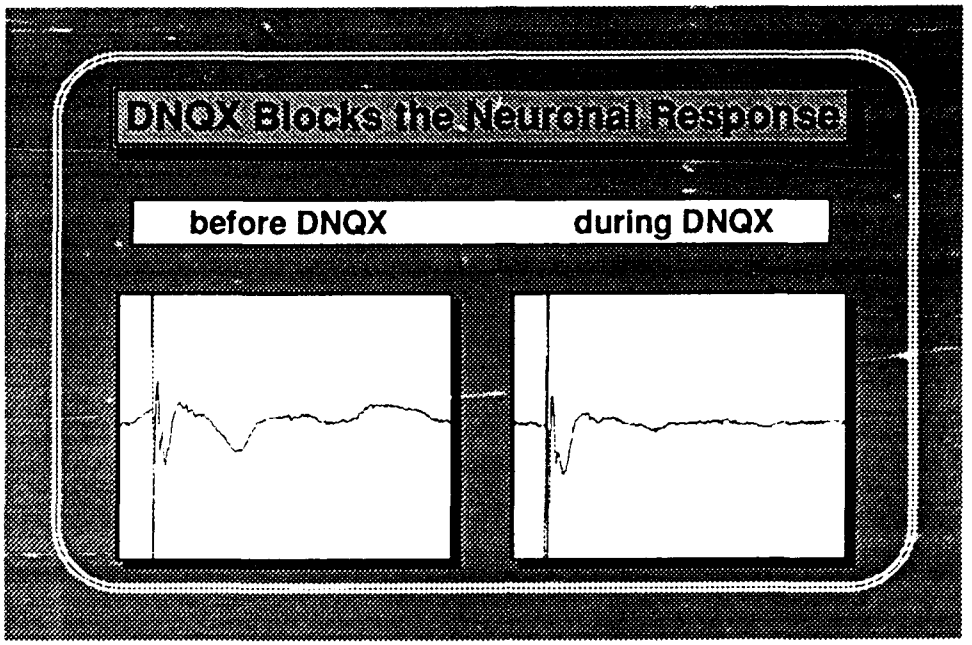
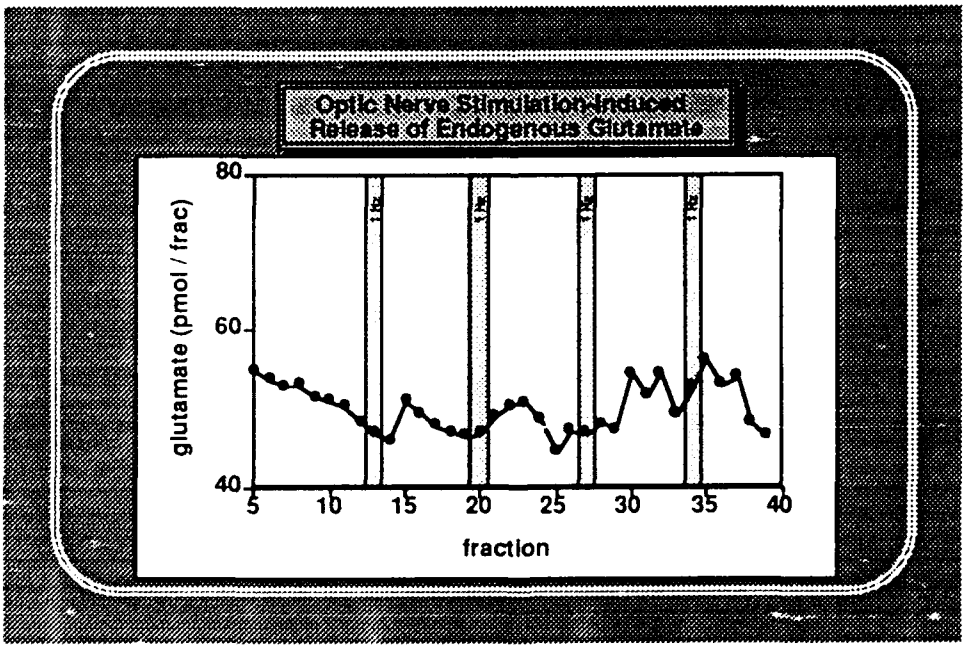
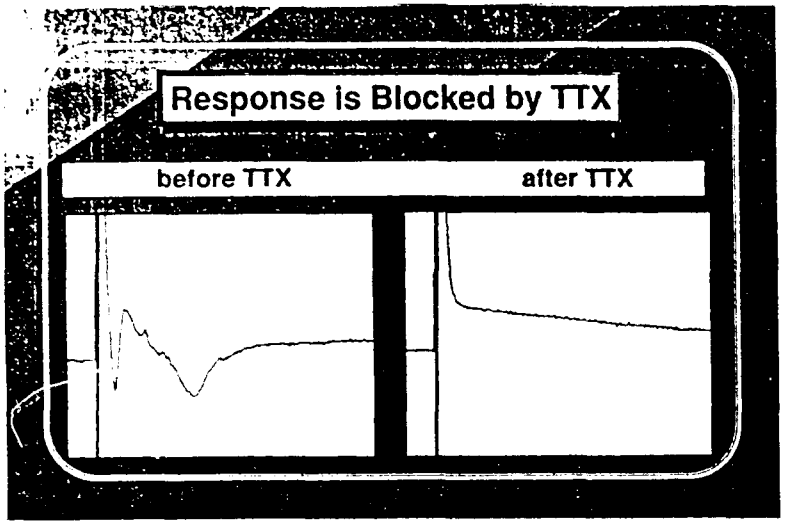
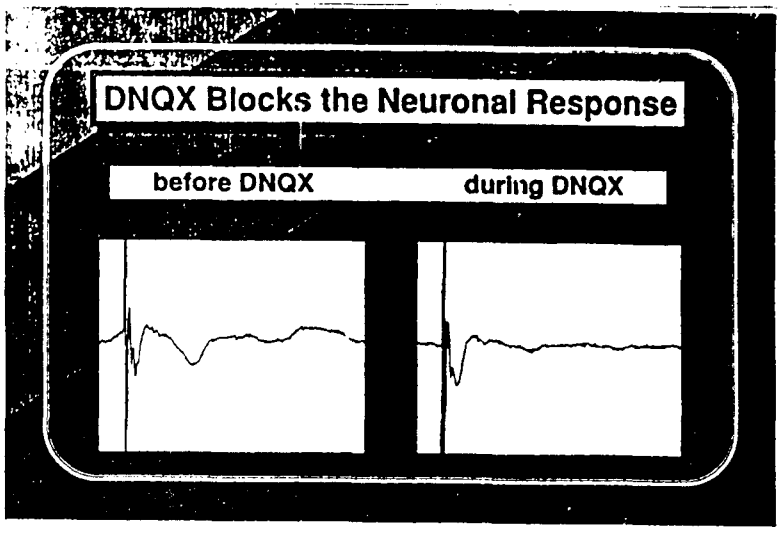
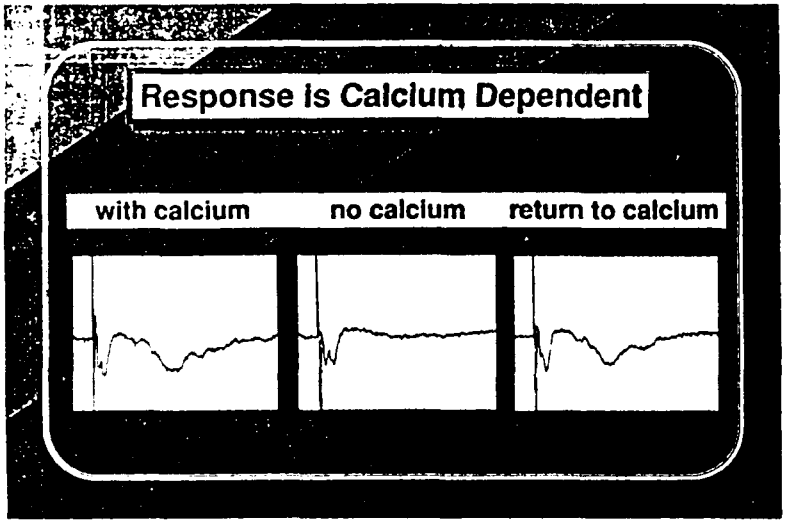
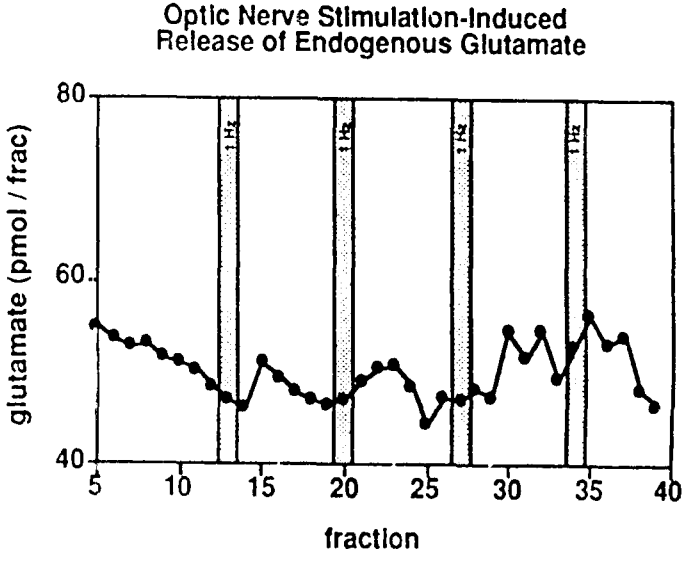

DNQX Blocks the Neuronal Response
before DNQX
during DNQX


Optic Nerve Stimulation-Induced Release of Endogenous Glutamate
glutamate (pmol / frac)
80
60
40
5
10
15
20
25
30
35
40
fraction
1 Hz
1 Hz
1 Hz
1 Hz









GILLETTE, Martha U.
Annual Technical Report, Year 1

RESEARCH ARTICLES PLANNED FOR PUBLICATION IN TECHNICAL JOURNALS

Medanic, M. and Gillette, M.U. Serotonin phase-shifts the rhythm of neuronal activity in the rat suprachiasmatic nucleus *in vitro*. *Journal of Physiology* In preparation for submission.

Tcheng, T.K. and Gillette, M.U. Localization of the circadian pacemaker in the suprachiasmatic nucleus and in microdissected subregions. *Journal of Physiology* More experiments are necessary before this is published.

PARTICIPATING PROFESSIONALS

Martha U. Gillette, P.I., Associate Professor of Cell & Structural Biology, and of Physiology. University of Illinois

Michael A. Rea, Co-P.I., Senior Scientist, USAF School of Aerospace Medicine, Brooks AFB, San Antonio, Texas

Marija Medanic, Graduate Research Assistant, Department of Physiology & Biophysics, University of Illinois; M.S. awarded in Jan., 1991; Pursuing Ph.D. currently.

Ann-Marie Michel, Research Specialist in Biological Science; working at USAF School of Aerospace Medicine; appointed through the University of Illinois

Thomas K. Tcheng, Graduate Research Assistant, Neuroscience Program, University of Illinois; currently taking Qualifying Examination for the Ph.D. program.

Eve A. Gallman, Postdoctoral Research Associate, Department of Cell & Structural Biology; University of Illinois

INTERACTIONS THROUGH MEETINGS AND COLLABORATIVE EXPERIMENTS

MEETINGS

Gillette, M.U. and Tcheng, T.K. 1990. Localization of a circadian pacemaker to the ventrolateral suprachiaasmatic nucleus (SCN). Presented at the Society for Research on Biological Rhythms, May, 1990. Amelia Island, FL

Tcheng, T.K. and Gillette, M.U. 1990. Electrical characterization of ventrolateral and dorsomedial regions of the suprachiasmatic nucleus. Presented at the Society for Neuroscience Meeting, October, 1990. St. Louis, MO

Medanic, M. and Gillette, M.U. 1990. Serotonin phase shifts the circadian rhythm of electrical activity in the rat SCN *in vitro*. Presented at the Society for Neuroscience Meeting, October, 1990. St. Louis, MO

Gillette, M.U. 1991. Cellular regulators of the SCN pacemaker studied in the brain slice. Presented as part of a panel, "Current Status of Circadian Rhythm Regulation in Mammals", at the 1991 Winter Conference for Brain Research at Vial, CO. Other members of the panel included L. Morin (SUNY-Stony Brook), D. Earnest (Rochester) and M. Lehman (Cinncinati).

COLLABORATIONS

With Dr. Mike Rea (USAF-SAM, Brooks AFB) we have explored a number of potential collaborative experiments. These included examining the expression of c-fos immunoreactive material in SCN brain slices before and after treatment with cGMP analog. The non-specific expression of fos seemed to be a major problem with the experiments as they were designed.

A second, more recent set of collaborative experiments involves elucidating the signal transduction pathway by which excitatory amino acids affect the SCN. These experiments were planned with Dr. Rea at the Winter Conference for Brain Research. We have begun by measuring cGMP levels in SCN from explants that have been stimulated with glutamate or NMDA. This experiment looks very promising and will be pursued in year 2. This work will be performed by Todd Weber, a Physiology & Biophysics graduate student in my laboratory, with tissue sent by Mike Rea. Todd Weber will also travel to Dr. Rea's lab to carry out some of this work under the tenure of his recently awarded Air Force Laboratory Research Fellowship.

SUMMARY OF PROGRESS

1) The preponderance of data suggest that the SCN pacemaker is distributed and is organized primarily in the VL-SCN, the region receiving afferent fibers from regulatory brain regions.

2) Serotonin is a potent regulator of the SCN. It induces phase-advances during the daytime portion of the circadian cycle only; at nighttime it is without effect when applied focally to the site that raphe afferents terminate. Serotonin appears to act through a $5HT^{1A}$ receptor.

3) Stimulation of the optic nerve under conditions that produce robust field potentials, that are reversibly blocked by TTX, causes release of ^{3}H-GLU and -ASP from the SCN region *in vitro*. Sources of variability in these results due to technical problems are being addressed.

AFOSR 90-0205

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October 28–November 2

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1st topic title BIOLOGICAL RHYTHMS & SLEEP topic number: 116

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2nd topic title _____ topic number: ___

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SEROTONIN PHASE SHIFTS THE CIRCADIAN RHYTHM OF ELECTRICAL ACTIVITY IN THE RAT SCN IN VITRO.

M. Medanic and M.U. Gillette, Dept. of Physiol. & Biophys. and Neuroscience Program, Univ. of Illinois, Urbana, IL 61801.

Ventrolateral (VL) regions of the suprachiasmatic nuclei (SCN) receive serotonergic projections from the raphe nuclei. We investigated the possible role of serotonin (5-HT) in the mammalian circadian system by examining its effect on the rhythm of electrical activity in the rat SCN *in vitro*.

Eight week old, male Long-Evans rats from our inbred colony, raised on 12L:12D schedule, were used. Hypothalamic brain slices containing the paired SCN were made during the day, and maintained *in vitro* for two days. A 30 μl drop of 10^{-6}M 5-HT was applied for 5 minutes to the VL region of one of the SCN at CT 7 (n=3), 13 (n=3) or 19 (n=4). The time of peak in the rhythm of neuronal activity was determined on the following day.

Exposure of the VL-SCN to 5-HT treatment during the subjective night (CT 13 and 19) did not significantly alter time-of-peak compared to untreated slices. However, treatment during the subjective day (CT 7) resulted in a 6.9±0.1 hr advance in the time-of-peak of neuronal activity in the next cycle. This suggests that the SCN are sensitive to 5-HT during the subjective daytime and respond with a phase advance in the circadian clock.

(Supported by AFORS grant 90-0205.)

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KEY WORDS: (see instructions pg. 4)

1. HYPOTHALAMUS
2. PACEMAKER
3. SUPRACHIASMATIC
4. RAPHE

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MARIJA MEDANIC — Printed or typed name
(217) 244-1842 — Telephone number

AFOSR-90-0205

Society for Research on Biological Rhythms: Abstract Form

Mailing Address of First Author DR. M Gillette Signature Martha U. Gillette
Dept. of Physiology & Biophysics - U of I Telephone (work) 217: 244-1355
524 Burrill Hall, 407 S Goodwin Ave. (home) 217: 367-9036
Urbana, IL 61801

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Society for Research on Biological Rhythms
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Northwestern University
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Evanston, Illinois 60208-3520 U.S.A. ***Abstracts must be postmarked by February 1, 1990.***

LOCALIZATION OF A CIRCADIAN PACEMAKER TO THE VENTROLATERAL SUPRACHIASMATIC NUCLEUS (SCN).

M.U. Gillette and T.K. Tcheng. Dept. of Physiol. & Biophys. and Neuroscience Program, Univ. of Illinois, Urbana, IL 61801.

The mammalian SCN contain an endogenous circadian pacemaker. Little is known of the pacemaker's intrinsic organization. Persistence of behavioral circadian rhythms after partial SCN lesions demonstrates that this structure need not be intact to drive circadian rhythms. The question arises, "Is the pacemaking function restricted to a particular region in the SCN, or is it distributed?" Anatomical and immunohistochemical studies of the SCN have revealed striking differences between neurons in the dorsomedial (DM) and ventrolateral(VL) SCN (van den Pol 1980, 1985). We hypothesize that the pacemaker is localized in one of these two regions.

In order to test this hypothesis, we prepared SCN in hypothalamic brain slices, surgically isolated progressively smaller regions of the SCN by microdissection, then examined ensemble neuronal activity for circadian rhythms (CRs) *in vitro*. Our previous work has shown that a 500 μm coronal slice from 2-5 mo Long-Evans rats, which contains less than the anterior-posterior extent of the SCN, produces a stable CR for at least 3 days *in vitro*. Trimming the slice to within 100 μm of the paired SCN results in an unperturbed CR. Neuronal activity in both the DM and VL regions in the intact slice peaks synchronously at CT 6.9 on day 2 (N=8).

Our current research further localizes the pacemaker. Bisecting the SCN by severing the commissure connecting the two nuclei has no apparent effect on the CR (N=4). Subdividing the bisected SCN into DM and VL halves results in a marked difference in CRs in these two regions. The VL region exhibits a peak in neuronal activity near CT 6.9 on day 2 (N=8). The DM-SCN does not exhibit a noticable peak in activity (N=6). These results support the hypothesis that the circadian pacemaker is localized, not distributed. Furthermore, they demonstrate that the VL-SCN contains a circadian pacemaker. Whether the DM-SCN contains a pacemaker whose electrical CR is uncoupled by the surgery remains to be determined.

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1st theme title: Neural Basis of Behavior theme letter: I
1st topic title Biological Rhythms and Sleep topic number: 116

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ELECTRICAL CHARACTERIZATION OF VENTROLATERAL AND DORSOMEDIAL REGIONS OF THE SUPRACHIASMATIC NUCLEUS.

T.K. Tcheng and M.U. Gillette. Neuroscience Program and Department of Physiology & Biophysics, University of Illinois, Urbana, IL 61801.

The rat suprachiasmatic nuclei (SCN) contain a circadian pacemaker that is expressed in the brain slice as a 24-hr oscillation in ensemble neuronal firing rate. We are studying neuronal firing patterns within the dorsomedial (DM) and ventrolateral (VL) SCN, the two major anatomical subdivisions, to further characterize the circadian pacemaker.

We have shown previously that hemisection of the SCN into DM and VL halves results in an unperturbed electrical circadian rhythm (ECR) in the VL-SCN and the possible loss of an ECR in the DM-SCN. Our current work elaborates upon this finding. In this study, the SCN are unequally divided, leaving less SCN in either the DM-SCN (DM-biased) or VL-SCN (VL-biased). Preliminary results suggest that after DM-biased hemisection the ECR in the DM-SCN is completely abolished (N=5). The effect of VL-biased hemisection on the ECR in the DM and VL regions is presently being examined. Additionally, we have observed that firing patterns of individual neurons change after hemisection. Firing patterns from control SCN and isolated VL and DM regions will be compared in order to identify firing patterns contributing to the ECR.

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KEY WORDS: (see instructions pg. 4)

1. Circadian
2. Rhythm
3. Pacemaker
4. Oscillator

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SEROTONERGIC AGONISTS ADVANCE THE CIRCADIAN RHYTHM OF NEURONAL ACTIVITY IN RAT SCN IN VITRO.

M. Medanic and M.U. Gillette. Dept. of Physiol. & Biophys. and Dept. of Cell & Struct. Biol., Univ. of Illinois, Urbana, IL 61801.

Serotonin (5-HT) directly affects the SCN pacemaker *in vitro*. Brief application of 5-HT to ventrolateral (VL) SCN during the subjective day phase-advances (ϕ_A) the time of peak neuronal activity with a maximal shift of 6.9±0.1 hr at CT 7. 5-HT is ineffective at night. This temporal sensitivity matches that for cAMP analogs. To confirm the specificity of 5-HT-induced ϕ_A and to investigate the mechanism by which 5-HT acts on the SCN, the effects of two 5-HT_1 agonists, 5-CT and 8-OH DPAT, were tested.

Hypothalamic brain slices containing the paired SCN were obtained from male Long-Evans rats (8wks old, raised in 12L:12D) and maintained *in vitro*. The slices were treated with 10^{-6}M 5-CT at CT 9 (n=3) or 15 (n=2), or with 10^{-6}M 8-OH DPAT at CT 9 (n=3) on day 1, by a 30 μl drop to the VL-SCN for 5 minutes. The time-of-peak in the rhythm of neuronal activity was accessed the following day.

While treatment of the VL-SCN with 5-CT at CT 15 did not significantly alter the rhythm, exposure at CT 9 resulted in a 6.0±0.1 ϕ_A of the time-of-peak. Similarly, administration of 8-OH DPAT at CT 9 induced a 6.9±0.1 ϕ_A of the peak time. This confirms the specificity of the 5-HT-induced ϕ_A and lends support to the hypothesis that 5-HT may affect the SCN via a 5-HT_1 receptor-linked pathway.

(Supported by AFORS grant 90-0205.)

KEY WORDS: (see instructions pg. 4)

1. SEROTONIN
2. PACEMAKER
3. HYPOTHALAMUS
4. CYCLIC AMP

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